

**WHAT IS CLAIMED IS:**

1. A method for producing an adenovirus comprising:

- a) growing host cells in media at a low perfusion rate;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells to produce a crude cell lysate;
- d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and
- f) reducing the concentration of contaminating nucleic acids in said  
crude cell lysate.

2. The method of claim 1, further comprising isolating an adenoviral particle from said cell lysate using chromatography.

3. The method of claim 1, wherein the glucose concentration in said media is maintained between about 0.7 and about 1.7g/L.

4. The method of claim 1, wherein said exchanging buffer involves a diafiltration step.

5. The method of claim 1, wherein said adenovirus comprises an adenoviral vector encoding an exogenous gene construct.

6. The method of claim 5, wherein said gene construct is operatively linked to a promoter.

5

7. The method of claim 6, wherein said promoter is SV40 IE, RSV LTR,  $\beta$ -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

8. The method of claim 1, wherein said adenovirus is a replication-incompetent adenovirus.

10

9. The method of claim 8, wherein the adenovirus is lacking at least a portion of the E1-region.

10. The method of claim 9, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.

15

11. The method of claim 1, wherein said host cells are capable of complementing replication.

12. The method of claim 1, wherein said host cells are 293 cells.

20

13. The method of claim 5, wherein said exogenous gene construct encodes a therapeutic gene.

14. The method of claim 13, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*  
5 antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, *zac1*, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase or p53.
- 10 15. The method of claim 14, wherein said therapeutic gene encodes p53.
16. The method of claim 1, wherein said cells are harvested and lysed *ex situ* using a hypotonic solution, hypertonic solution, freeze-thaw, sonication, impinging jet, microfluidization or a detergent.
- 15 17. The method of claim 1, wherein said cells are harvested and lysed *in situ* using a hypotonic solution, hypertonic solution, or a detergent.
18. The method of claim 17, wherein said cells are lysed and harvested using  
20 detergent.
19. The method of claim 18, wherein said detergent is Thesit<sup>®</sup>, NP-40<sup>®</sup>, Tween-20<sup>®</sup>, Brij-58<sup>®</sup>, Triton X<sup>®</sup>-100 or octyl glucoside.

20. The method of claim 1, wherein said lysis is achieved through autolysis of infected cells.
- 5 21. The method of claim 1, wherein said cell lysate is treated with Benzonase<sup>®</sup>, or Pulmozyme<sup>®</sup>.
22. The method of claim 2, wherein said isolating consists essentially of a single chromatography step.
- 10 23. The method of claim 22, wherein said chromatography step is ion exchange chromatography.
24. The method of claim 23, wherein said ion exchange chromatography is anion  
15 exchange chromatography.
25. The method of claim 24, wherein said anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.
- 20 26. The method of claim 24, wherein said anion exchange chromatography utilizes Toyopearl Super Q 650M, MonoQ, Source Q or Fractogel TMAE.

27. The method of claim 24, wherein said ion exchange chromatography is carried out at a pH range of between about 7.0 and about 10.0.

28. The method of claim 1, further comprising a concentration step employing membrane filtration.

29. The method of claim, 28, wherein said filtration is tangential flow filtration.

30. The method of claim, 28, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.

31. An adenovirus produced according to a process comprising the steps of:

- a) growing host cells in media at a low perfusion rate;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells to produce a crude cell lysate;
- d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and
- f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

32. The adenovirus of claim 31, wherein adenovirus comprises an adenoviral vector encoding an exogenous gene construct.

33. The adenovirus of claim 31, wherein said gene construct is operatively linked to a promoter.

5 34. The adenovirus of claim 31, wherein said adenovirus is a replication-incompetent adenovirus.

35. The adenovirus of claim 34, wherein said adenovirus is lacking at least a portion of the E1-region.

10

36. The adenovirus of claim 31, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.

37. The adenovirus of claim 31, wherein said host cells are capable of complementing  
15 replication.

38. The adenovirus of claim 31, wherein said host cells are 293 cells.

20

39. The adenovirus of claim 31, wherein said exogenous gene construct encodes a therapeutic gene.

40. The adenovirus of claim 39, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense

*jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*  
antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, *zac1*,  
*scFV ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1,  
FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10,  
IL-11 IL-12, GM-CSF G-CSF, thymidine kinase or p53.

41. The adenovirus of claim 40, wherein said therapeutic gene is p53.
42. The adenovirus of claim 33, wherein said promoter is SV40 IE, RSV LTR,  $\beta$ -actin or CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.
43. A method for the purification of an adenovirus comprising:
  - a) growing host cells;
  - b) infecting said host cells with an adenovirus;
  - c) harvesting and lysing said host cells by contacting said cells with a detergent to produce a crude cell lysate;
  - d) concentrating said crude cell lysate;
  - e) exchanging buffer of crude cell lysate; and
  - f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

44. The method of claim 43, further comprising isolating an adenoviral particle from said lysate using chromatography.

5 45. The method of claim 43, wherein said host cells are grown in media wherein a glucose concentration is maintained between about 0.7 and about 1.7g/L.

46. The method of claim 43, wherein said exchanging buffer involves a diafiltration step.

10 47. The method of claim 43, wherein said detergent is Thesit<sup>®</sup>, NP-40<sup>®</sup>, Tween-20<sup>®</sup>, Brij-58<sup>®</sup>, Triton X-100<sup>®</sup> or octyl glucoside.

48. The method of claim 47, wherein said detergent is present in the lysis solution at a concentration of about 1% (w/v).

15

49. The method of claim 43, wherein said isolating consists essentially of a single chromatography step.

20

50. The method of claim 44, wherein said chromatography step is ion exchange chromatography.

51. An adenovirus produced according to a process comprising the steps of:



- a) growing host cells;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells by contacting said cells with a detergent to produce a crude cell lysate;
- d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and
- f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

52. A method for the purification of an adenovirus comprising:

- a) growing host cells in serum-free media;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells to produce a crude cell lysate;
- d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and
- f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

53. The method of claim 52, wherein said host cells are adapted for growth in serum-free media.

54. The method of claim 52, wherein said cells are grown as a cell suspension culture.

55. The method of claim 52, wherein said cells are grown as an anchorage-dependent culture.

5 56. The method of claim 53, wherein said adaptation for growth in serum-free media comprises a sequential decrease in the fetal bovine serum content of the growth media.

10 57. The method of claim 53, wherein said serum-free media comprises a fetal bovine serum content of less than 0.03% v/v.

58. The method of claim 52, further comprising isolating an adenoviral particle from said lysate using chromatography.

15 59. The method of claim 52, wherein said lysis is achieved through autolysis of infected cells.

60. The method of claim 52, wherein said exchanging buffer involves a diafiltration step.

20 61. The method of claim 52, wherein said detergent is Thesit<sup>®</sup>, NP-40<sup>®</sup>, Tween-20<sup>®</sup>, Brij-58<sup>®</sup>, Triton X-100<sup>®</sup> or octyl glucoside.

62. The method of claim 52, wherein said detergent is present in the lysis solution at a concentration of about 1% (w/v).

5 63. The method of claim 52, wherein said isolating consists essentially of a single chromatography step.

64. The method of claim 58, wherein said chromatography step is ion exchange chromatography.

10 65. An adenovirus produced according to a process comprising the steps of:

- a) growing host cells in serum-free media;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells to produce a crude cell lysate;
- 15 d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and
- f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

20 66. A 293 host cell adapted for growth in serum-free media.

67. The cell of claim 66, wherein said cell is adapted for growth in suspension culture.

68. The cell of claim 66, wherein the cell is deposited with the ATCC and is designated as a IT293SF cell.
- 5 69. The cell of claim 66, wherein said adaptation for growth in serum-free media comprises a sequential decrease in the fetal bovine serum content of the growth media.